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Project No. _____

Book No. _____

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TITLE CN65 HindIII partial

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AGENDA

- I. Characterization of PSE - Eric, Lena, Joe
5' end and 3' end of PSE defined within 200 bp
ARE within the Cla I site required
Sequencing
Thus, at least three elements: ARE, Left and Right
within PSE
Gel Shift with 200 bp segments across PSE - Eric
Required proteins
Required and tissue-specific proteins
- II. Transgenic Mice Constructs - Gail, Dan
1. 8kb driving β -gal (need 6 kb in CN70, β -gal in BS)
2. minimal enhancer driving β -gal (HindIII PSE in CN70)
3. 6kb driving DT-A (CN45)
- III. Adenovirus Constructs - Gail, Lena
1. PSE minimal enhancer driving β -gal in Δ E1sp1A and 1B
2. PSE minimal enhancer driving CAT in Δ E1sp1A and 1B
3. PSE minimal enhancer driving DT-A in Δ E1sp1A and 1B
4. CMV driving HSV-tk in Δ E1sp1A and 1B
5. CMV driving cytosine deaminase in Δ E1sp1A and 1B
6. CMV driving β -gal in Δ E1sp1A
What seems to be the problem with subcloning? Special Meeting
- IV. Construction of Adenovirus vector: Δ E1sp1A, Δ E1sp1B, BHG10, BHG11, BHGE3, FG140, pXC1, pABS.4 -Dan
1. plaque assay
2. infectious DNA
3. homologous recombination to make infectious DNA
4. new Ad5 recombinant viruses
- V. Tissue Specificity of PSE - In Vivo - Eric, Joe, Lena
repeated?
Direct Injection of DNA into Tissues and Tumors
- VI. Liposomes - Henry
Experiments

Synthesizing oligos for linked PCR

passed cells into 6-well
20 x 60mm plates

Did again analysis of yesterday's
transfections - p15.131

Eric's CN65 limit HindIII

failed - no colonies

Eric p18.21 used 10 μ l of 0.5 μ g/ μ l

in a 20 μ l rxn: 5.0 μ l 0.05

units of enzyme, 15' 22°C

- best incubation 65°C x 20'

and run 1 μ l on gel

- ARE for 1V. 2 lanes each 1 μ l

10 mM AMPES 8.7 μ l 15'

- And come to 25 μ l

- heat inactivate 75°C x 10'

- run gel

- take to band, gave clean

- digest

- limit

- Eric got no (more) colonies. I will

leave -> to prep gel step, 6-11-91

Best run analyzed gel of digest

✓ 10 μ l pna

✓ 2 μ l NEB 2

✓ 2 μ l 10 x BSA

✓ 5 μ l H₂O

✓ 1 μ l enzyme (S, S, 0.05 units)

To: 1.57 P.M.

and 2.05 P.M. ✓

20' x 65°C ✓

heat spin ✓

1 μ l 10 mM AMPES ✓

1 μ l 5.0 μ l 0.05 ✓

To: 2.46 P.M.

1.25 μ l 0.5 M EDTA ✓

75°C 20' ✓

10 μ l 3M NaCl 2 μ l 0.05 ✓

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Witnessed & Understood by me, _____

Date _____

Invented by _____

Recorded by _____